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(54) Title: MAMMALIAN CELLS EXPRESSING A HYBRID RECEPTOR			
(57) Abstract			
<p>Mammalian cells containing a hybrid DNA insert which comprises a first DNA sequence encoding part of the extracellular domain of a first cellular receptor and a second DNA sequence encoding part of the extracellular domain of second cellular receptor which is specific for a different ligand than the first cellular receptor have favourable growth properties in serum-containing as well as serum-free media.</p>			

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MAMMALIAN CELLS EXPRESSING A HYBRID RECEPTOR

FIELD OF INVENTION

The present invention relates to a cell expressing a hybrid between different cellular receptors and a process of producing a desired polypeptide from the cell.

BACKGROUND OF THE INVENTION

Cell culture has been carried out for many years in order to investigate for instance the physiology of mammalian cells and the relationship between mammalian cells and other organisms such as viruses as well as the impact of a large number of different compounds on the cells. The equipment traditionally used for cell culture includes glass vessels, petri dishes, T-flasks and the like. The media formulations used for cell culture experiments have been named for the persons who developed them (often on a trial-and-error basis) or modified them in various ways, i.a. Ham, Dulbecco, Leibovitz, McCoy, Waymouth, Eagle, Iscove, etc.

These conventional media all contain serum which was found to make the cells grow at a reasonable rate for experimentation purposes, and the serum which has generally been found to provide the best growth rate is foetal calf serum. The scarcity and variable quality of this serum has created serious problems for cell culture in that the experiments have often been difficult to reproduce.

Although other aspects of cell culture, in particular the equipment used to culture the cells on a larger scale, have undergone a rapid development in recent years, the composition of the standard media used for cultivation is substantially the same as in the early days of cell culture. Thus, the media still have a high serum content.

Modern concepts of producing pharmaceuticals on the basis of cell culture require a stringent process control, not only with respect to purification, but also with respect to culture conditions. The use of serum in media is inconsistent with this requirement: due to its biological nature, the composition of serum varies from batch to batch resulting in variability of the cultivation process. Serum also represents the single most important source of unrelated protein present in the culture liquid, which can only be removed from the product of interest by laborious methods resulting in a lower yield of the product of interest. Moreover, serum is the single most important source of contamination of the cell culture with mycoplasma, bacteria or viruses, and costly as well as time-consuming measures have to be taken to remove these contaminants from the serum and to test for their absence.

It would therefore be highly desirable to omit serum from media used for mammalian cell culture. Attempts have been made to adapt cell lines already used in the production of pharmaceuticals or potentially useful cell lines to growth in serum-free media or media with a reduced serum content. It has, however, been observed that when adaptations to serum-free or low serum media have been made, this has been at the expense of growth performance, i.e. the cells grow at a slower rate than in serum-containing media, resulting in lower productivity and higher susceptibility to invading contaminants.

A cell line which is able to grow at an increased growth rate than normally observed in serum-containing media, not only in serum-containing media, but also in serum-free media would therefore be of great potential interest for cell culture in the pharmaceutical industry. By omitting serum, it would be simpler to purify the product of interest resulting in improved yields and purity, and a stable faster cell growth rate would lead to a high process productivity, i.e. because rapid-

growing cells would be less susceptible to infectious organisms.

SUMMARY OF THE INVENTION

It has surprisingly been found that cells with favourable growth properties in serum-containing as well as serum-free media may be obtained by introducing into the cells a DNA construct which comprises a hybrid DNA sequence coding for parts of two different cell surface receptors.

Accordingly, the present invention relates to a mammalian cell containing a hybrid DNA insert which comprises a first DNA sequence encoding part of the extracellular domain of a first cellular receptor and a second DNA sequence encoding part of the extracellular domain of a second cellular receptor which is specific for a different ligand than the first cellular receptor.

Cellular receptors which are typically located on cell surfaces mediate certain important cell to cell interactions involving the transmission of extracellular signals by the interaction with ligands. Such receptors are composed of an extracellular domain (in monomeric or dimeric form) which is capable of specifically recognizing and binding a particular ligand and which may have highly glycosylated and protease-resistant structure, a transmembrane domain which is responsible for anchoring the receptor in the cell membrane and which consists of a hydrophobic sequence of some 25 amino acids, and a cytoplasmic domain which is responsible for generating a cellular signal as a response to the binding of the ligand to the extracellular domain; the cytoplasmic domain(s) may define an enzymatic activity which is triggered by ligand binding.

The reason why hybrid receptors of the invention may promote the growth of cells (particularly in serum-free media) has not

yet been determined. It is, however, currently assumed that such hybrid receptor constructions provide a constant stimulation of the growth regulatory system of the cells so that their dependence on growth factors (e.g. those present in serum) is reduced. Furthermore, it is possible that hybrid receptors are less sensitive to non-activating ligands (e.g. degradation products or metabolites from the cells) and therefore less likely to be inhibited for binding than the natural receptor.

A number of naturally occurring receptors have previously been identified. Thus, Rubin et al., J. Immun. **135**, 1985, pp. 3172-3177, describe the release of large quantities of the interleukin-2 receptor (IL-2-R) into the culture medium of activated T-cells. The DNA sequence coding for the insulin receptor has been published by A. Ullrich et al., Nature **313**, 28 Feb. 1985, pp. 756-761, and S. Seino, Proc. Natl. Acad. Sci. USA **86**, 1989, pp. 114-118. Similarly, the DNA sequence coding for the IGF-I receptor has been published by A. Ullrich et al., The EMBO J. **5**(10), 1986, pp. 2503-2512.

European Patent Application, Publication No. 244 221 discloses receptors which are hybrids between the extracellular ligand-binding domain of one receptor fused to a heterologous reporter polypeptide which may be the cytoplasmic domain of another receptor.

B. Kobilka et al, Science **240**, 3 June 1988, pp. 1310-1316, disclose chimeras between $\alpha 2$ and $\beta 2$ adrenergic receptors constructed by replacing DNA sequences encoding various parts of the membrane-spanning domain of one of the receptors by the DNA sequences encoding corresponding parts of the other receptor and expressing the sequences in Xenopus laevis oocytes.

I. Lax et al., The EMBO J. **8**(2), 1989, pp. 421-427, disclose chimeras between chicken and human EGF receptors constructed by replacing DNA sequences encoding various parts of one of the

receptors by DNA sequences encoding the corresponding parts of the other receptor, and expressed on the surface of mammalian cells.

However, none of these prior publications discloses any use of either natural or hybrid receptors for promoting the growth of mammalian cells.

In the present context, the expression "specific for a different ligand" is intended to indicate that the second receptor specifically binds a ligand which, in a given organism, has a different biological function and/or activity than the ligand binding to the first receptor. The expression is intended to distinguish the present hybrid receptors from those described by, for instance, I. Lax et al., op. cit., which are hybrids of receptors specific for essentially the same ligand derived from two different organisms. The term "ligand" may be defined as a substance which, in nature, is capable of binding to a particular cellular receptor. Preferred ligands are those which act in a similar way as the natural ligand for the receptor in question (e.g. a hormone, growth factor, cytokine or cell adhesion molecule).

DETAILED DISCLOSURE OF THE INVENTION

In particular, the DNA insert introduced into the cell of the present invention is one in which the first DNA sequence (encoding part of the extracellular domain of the first receptor) encodes an exon or a fragment thereof, or in which the second DNA sequence (encoding part of the extracellular domain of the second receptor) encodes an exon or a fragment thereof. For the present purpose, either the first or the second DNA sequence may encode a ligand-binding site of the first or second receptor.

In a particularly preferred embodiment of the cell of the present invention, the DNA insert is one in which the DNA

sequences coding for the first and second receptors exhibit a high degree of homology in the organization and/or structure of their exons. This may be advantageous as it is possible to systematically substitute specific fragments from one receptor for the corresponding fragments from the other receptor. When producing a cell of the invention, it may therefore be an advantage to initially replace one or more exons from one of the receptors by the corresponding exon or exons from the other receptor, and to test the growth properties of cells into which the DNA construct coding for each hybrid has been introduced.

In this embodiment of the DNA insert introduced in the cell of the invention, a DNA sequence encoding one or more exons, or a fragment thereof, of the extracellular domain of the first receptor may be replaced by a DNA sequence encoding the corresponding exon or exons, or fragment thereof, of the extracellular domain of the second receptor. Alternatively, a DNA sequence encoding one or more exons, or a fragment thereof, of the extracellular domain of the second receptor may be replaced by a DNA sequence encoding the corresponding exon or exons, or fragment thereof, of the extracellular domain of the first receptor.

As indicated above, the parent receptors are cell surface receptors, in particular receptors for hormones, growth factors, cytokines or cell adhesion molecules. Thus, the receptors may be selected from the group consisting of the insulin, insulin-like growth factor (IGF), platelet-derived growth factor (PDGF), epidermal growth factor (EGF), transforming growth factor (TGF), including TGF- α and TGF- β , growth hormone and prolactin receptors. The hybrid receptor may advantageously be one comprising at least part of the binding site for a ligand produced by the cell itself (e.g. containing the whole or part of the EGF binding site of the EGF receptor). This permits the cell to stimulate its own growth, thereby reducing the need for adding external growth promoting agents to the culture medium in order to stimulate cell growth.

In the above-described preferred embodiment of the DNA insert of the invention, hybrids are constructed from receptors which exhibit the aforementioned homology in the overall organization of their DNA.

5 More specifically, in the DNA insert introduced in the cell of the invention, the DNA sequence coding for exon 2, or a fragment thereof, of the insulin receptor may be replaced by the DNA sequence coding for exon 2, or a fragment thereof, of the IGF receptor. Alternatively, the DNA sequence coding for
10 exon 3, or a fragment thereof, of the insulin receptor may be replaced by the DNA sequence coding for exon 3, or a fragment thereof, of the IGF receptor. In a further alternative embodiment, the DNA sequence coding for exons 2 and 3, or a fragment thereof, of the insulin receptor may be replaced by
15 the DNA sequence coding for exons 2 and 3, or a fragment thereof, of the IGF receptor.

In another embodiment of the DNA insert, the DNA sequence coding for exon 2, or a fragment thereof, of the IGF receptor may be replaced by the DNA sequence coding for exon 2, or a
20 fragment thereof, of the insulin receptor. Alternatively, the DNA sequence coding for exon 3, or a fragment thereof, of the IGF receptor may be replaced by the DNA sequence coding for exon 3, or a fragment thereof, of the insulin receptor. As a
25 further alternative, the DNA sequence coding for exons 2 and 3, or a fragment thereof, of the IGF receptor may be replaced by the DNA sequence coding for exons 2 and 3, or a fragment thereof, of the insulin receptor.

Similarly, a DNA sequence coding for exon 1 (preferably combined with exon 2 or a fragment thereof) or one or more of
30 exons 4-11 or fragments thereof of the insulin receptor may be replaced by the corresponding DNA sequence from the IGF receptor, or vice versa.

One example of a cell of the invention exhibiting favourable growth properties is one in which a DNA insert has been introduced, which has the partial DNA sequence shown in Fig. 4A-4F (encoding the extracellular domain of the hybrid receptor), or a suitable modification thereof. Suitable modifications of the DNA sequence may comprise nucleotide substitutions which do not give rise to another amino acid sequence of the hybrid polypeptide, but which facilitate the production of the polypeptide, or nucleotide substitutions which do give rise to a different amino acid sequence of the hybrid polypeptide. Other possible modifications may be insertion of one or more nucleotides into the sequence, addition of one or more nucleotides at either end of the sequence and deletion of one or more nucleotides at either end of or within the sequence.

When the first receptor is the PDGF receptor, the second receptor may suitably be the EGF receptor or vice versa. When the first receptor is the EGF receptor, the second receptor may also be the TGF (α or β) receptor, and vice versa. When the first receptor is the growth hormone receptor, the second receptor may suitably be the prolactin receptor or vice versa.

The DNA insert encoding the hybrid receptor may be prepared synthetically by established standard methods, e.g. the phosphoamidite method described by S.L. Beaucage and M.H. Caruthers, Tetrahedron Letters **22**, 1981, pp. 1859-1869, or the method described by Matthes et al., EMBO Journal **3**, 1984, pp. 801-805. According to the phosphoamidite method, oligonucleotides are synthesized, e.g. in an automatic DNA synthesizer, purified, annealed, ligated and cloned in suitable vectors.

The DNA insert may also be of genomic or cDNA origin, for instance obtained by preparing a genomic or cDNA library and screening for DNA sequences coding for all or part of the hybrid receptor polypeptide by hybridization using synthetic oligonucleotide probes in accordance with standard techniques (cf. Sambrook et al., Molecular Cloning: A Laboratory Manual,

2nd Ed., Cold Spring Harbor, 1989). In this case, a genomic or cDNA sequence encoding a part of either receptor may be modified at a site corresponding to the site(s) at which it is desired to introduce amino acid substitutions, e.g. by site-directed mutagenesis using synthetic oligonucleotides encoding the desired amino acid sequence for homologous recombination in accordance with well-known procedures.

Finally, the DNA insert may be of mixed synthetic and genomic, mixed synthetic and cDNA or mixed genomic and cDNA origin prepared by annealing fragments of synthetic, genomic or cDNA origin (as appropriate), the fragments corresponding to various parts of the entire DNA insert, in accordance with standard techniques. The DNA insert may also be prepared by polymerase chain reaction using specific primers, for instance as described in US 4,683,202.

The hybrid DNA insert may be introduced into the cell by transfection of the cell with a recombinant expression vector comprising the insert. The expression vector may be any vector which may conveniently be subjected to recombinant DNA procedures, and the choice of vector will often depend on the host cell into which it is to be introduced. Thus, the vector may be an autonomously replicating vector, i.e. a vector which exists as an extrachromosomal entity, the replication of which is independent of chromosomal replication, e.g. a plasmid. Alternatively, the vector may be one which, when introduced into a host cell, is integrated into the host cell genome and replicated together with the chromosome(s) into which it has been integrated.

In the vector, the hybrid DNA insert should be operably connected to a suitable promoter sequence. The promoter may be any DNA sequence which shows transcriptional activity in the host cell of choice and may be derived from genes encoding proteins either homologous or heterologous to the host cell. Examples of suitable promoters for directing the transcription

of the DNA insert in mammalian cells are the SV 40 promoter (Subramani et al., Mol. Cell Biol. 1, 1981, pp. 854-864), the MT-1 (metallothionein gene) promoter (Palmiter et al., Science 222, 1983, pp. 809-814) or the adenovirus 2 major late promoter.

The DNA insert should also be operably connected to a suitable terminator, such as the human growth hormone terminator (Palmiter et al., op. cit.). The vector may further comprise elements such as polyadenylation signals (e.g. from SV 40 or 10 the adenovirus 5 Elb region), transcriptional enhancer sequences (e.g. the SV 40 enhancer) and translational enhancer sequences (e.g. the ones encoding adenovirus VA RNAs).

The recombinant expression vector may also comprise a DNA sequence enabling the vector to replicate in the host cell in 15 question. An examples of such a sequence is the SV 40 origin of replication. The vector may also comprise a selectable marker, e.g. a gene the product of which complements a defect in the host cell, such as the gene coding for dihydrofolate reductase (DHFR) or one which confers resistance to a drug, e.g. neomycin, hygromycin or methotrexate. 20

The procedures used to ligate the hybrid DNA insert with the DNA sequences coding for the promoter and the terminator, respectively, and to insert them into suitable vectors containing the information necessary for replication, are well 25 known to persons skilled in the art (cf., for instance, Sambrook et al., op.cit.).

The cell of the present invention may suitably be used for the production of proteins of polypeptides of interest. Thus, the cell may further contain an inserted DNA sequence encoding a 30 desired polypeptide. Examples of such polypeptides are the human blood clotting factors IX, VIII or VII, human tissue-type plasminogen activator, human protein C, human plasminogen, etc.

Examples of suitable mammalian cells for transfection with expression vector containing the DNA insert described above are the COS (ATCC CRL 1650), BHK (ATCC CRL 1632, ATCC CCL 10) NIH/3T3 (ATCC CRL 1658) or CHO (ATCC CCL 61) cell lines. 5 Methods of transfecting mammalian cells and expressing DNA sequences introduced in the cells are described in e.g. Kaufman and Sharp, J. Mol. Biol. 152, 1982, pp. 601-621; Southern and Berg, J. Mol. Appl. Genet. 1, 1982, pp. 327-341; Loyter et al., Proc. Natl. Acad. Sci. USA 79, 1982, pp. 422-426; Wigler et al., Cell 14, 1978, p. 725; Corsaro and Pearson, Somatic Cell Genetics 7, 1981, p. 603, Graham and van der Eb, Virology 52, 1973, p. 456; and Neumann et al., EMBO J. 1, 1982, pp. 841-845.

In another aspect, the present invention relates to a process 15 for producing a desired polypeptide, which comprises culturing a cell as described above in a suitable nutrient medium under conditions which are conducive to the expression of the polypeptide, and recovering the polypeptide from the culture. Although the medium used to culture the cells may be any 20 conventional medium suitable for growing mammalian cells, the cell of the invention has surprisingly been found to exhibit exceptionally favourable growth properties in serum-free medium which, as indicated above, is an advantage, i.e. because the purification of the desired polypeptide will be simplified, and 25 the yield of the polypeptide will be improved.

The polypeptide produced by the cells in this manner will often be secreted to the growth medium and may be recovered from the medium by conventional procedures including separating the host cells from the medium by centrifugation or filtration, precipitating the proteinaceous components of the supernatant or filtrate by means of a salt, e.g. ammonium sulphate, followed 30 by purification by a variety of chromatographic procedures, e.g. ion exchange chromatography, affinity chromatography, or the like.

BRIEF DESCRIPTION OF THE DRAWINGS

The invention is further described with reference to the drawings, wherein

Fig. 1A-1I shows the full-length cDNA sequence of the human insulin receptor;

Fig. 2 shows the assembly into the α -subunit and part of the β -subunit of the IGF-I receptor (sIGF-I-R) of cDNA fragments generated by reverse transcription and PCR using specific oligonucleotide primers; the circled numerals refer to the primers listed in Example 1;

Fig. 3A-3H shows the cDNA sequence of the soluble IGF-I receptor and the deduced amino acid sequence thereof, indicated in the conventional one-letter code;

Fig. 4A-4I shows the cDNA sequence of the extracellular domain of a hybrid insulin/IGF-I receptor, wherein exons 2 and 3 of the insulin receptor have been replaced by exons 2 and 3 of the IGF-I receptor, and the deduced amino acid sequence thereof, indicated in the conventional one-letter code;

Fig. 5 is a graph showing the growth curves (day 3-7) of three different cell lines, BHK, FCW 38-16 (BHK transfected with DNA encoding the hybrid insulin/IGF-I receptor according to Example 1) and hIR 12-14 (BHK transfected with DNA coding for the wild-type human insulin receptor), in medium containing 5% foetal calf serum;

Fig. 6 is a graph showing the growth curves (day 1-3) of three different cell lines, BHK, FCW 38-16 (BHK transfected with DNA encoding the hybrid insulin/IGF-I receptor according to Example 1) and hIR 12-14 (BHK transfected with DNA coding for the wild-

type human insulin receptor), in medium containing 5% foetal calf serum; and

Fig. 7 is a graph showing the growth curves (day 1-8) of three different cell lines, BHK, FCW 38-16 (BHK transfected with DNA encoding the hybrid insulin/IGF-I receptor according to Example 1) and hIR 12-14 (BHK transfected with DNA coding for the wild-type human insulin receptor), in serum-free medium supplemented with human insulin (BHK cells were also grown in serum-free medium without any added human insulin).

10 The invention is further illustrated in the following examples which are not in any way intended to limit the scope of the present invention.

EXAMPLE 1

Construction of cDNA encoding the human insulin receptor

15 Insulin receptor cDNA was isolated from a cDNA library generated from poly(A)⁺ mRNA isolated from the human lymphoblastoid cell line IM9 (available from the American Type Culture Collection, Rockville, Maryland, under the catalogue number ATCC CCL 159) stimulated with 1.4 µg/ml cortisol for 20
20 hours, substantially according to the method described by Okayama and Berg, Mol. Cell Biol. 2, 1982, p. 161 ff.; Okayama and Berg, Mol. Cell Biol. 3(2), Feb. 1983, pp. 280-289; and Noma et al., Nature 319, 1986, p. 640 ff. An approximately 4000 bp clone containing the 3' end of the insulin receptor was
25 isolated. To obtain a full-length clone, primer extension was applied on mRNA from the IM9 cells using (10 µg total mRNA) AMV reverse transcriptase (60 U, available from Pharmacia LKB Biotechnology, Sweden) with 800 ng of the 3' oligonucleotide primer 5'-CATCTCAGCAAGATCTTGTC-3'. The second strand was
30 synthesized as described by Okayama and Berg, op. cit., using the 5' oligonucleotide 5'-ACCGGGAGCGCGCTCTGATC-3' as primer. The cDNA was digested with the restriction endonucleases BssHII

and BglII and ligated into an appropriate vector. A 1550 bp clone containing the 5' end of the insulin receptor was isolated. The full-length insulin receptor was assembled in the mammalian expression vector Zem219b (described in DK Patent 5 Application No. 3023/88) digested with NcoI and XbaI by ligating the 5' cDNA clone digested with NcoI and BglII and the 3' cDNA clone digested with SpaI and BglII. The DNA sequence of the full length insulin receptor cDNA is shown in Fig. 1A-1F.

Construction of cDNA encoding a soluble IGF-I receptor

10 A soluble IGF-I receptor cDNA was prepared from mRNA from human term placenta by a method involving polymerase chain reaction (PCR) using specific oligonucleotide primers (cf. R.K. Saiki et al., Science 239, 29 Jan., 1988, pp. 487-491, and US 4,683,202). mRNA was prepared by standard procedures as 15 described in Sambrook et al., op. cit. The mRNA was reverse transcribed into cDNA with AMV reverse transcriptase (Pharmacia LKB Biotechnology, Sweden) using the appropriate specific primers or an oligo-dT-primer at a final concentration of 800 ng/3.5 µg mRNA.

20 IGF-I receptor cDNA fragments were amplified by PCR using the Gene Amp kit (Perkin Elmer Cetus, Norwalk, CT, USA) as recommended by the manufacturer.

In each PCR, approximately 1 µg of the reverse transcribed mRNA was used as template. The primers used for PCR all contained an 25 endonuclease restriction site permitting subcloning and assembly of the soluble IGF-I receptor. the following specific primers were used for PCR

1. 5'-CCA AAT AGG ATC CAT GAA CTC TGG CTC CGG AGG-3'
2. 3'-CCC GAA GTA GGC CTT AAG GTC GGT CTC GT-5'
- 30 3. 5'-TGG GCA GCT GCA GCG CGC CTG-3'
4. 3'-ATT GGA CCG TGG CCA TGG CCG-5'
5. 5'-TAA CCT GGC ACC GGT ACC GGC-3'

6. 3'-TCA AAG AGT TGC TTC GAA GAC-5'
7. 5'-AAC ACC ACG GCC GCA GAC ACC-3'
8. 3'-TGT CCT ATA CTT TTG ATT AGA TCT GAC TAG T-5'

The following restriction sites native to the IGF-I receptor were part of the PCR primers: PstI, Asp718, XmaIII and HindIII. A BamHI site was incorporated in the primer 1 used to generate the 5' end of the receptor cDNA by introducing a mismatch in the sequence. The primer 8 used to generate the 3' end of the soluble IGF-I receptor cDNA was designed by introducing a mismatch in the sequence so as to include a termination codon in nucleotide position 2842-2844 followed by an XbaI site. Each PCR reaction cycle comprised denaturation of the template at 94°C for 1 minute, and annealing of the primers to the templates for 2 minutes at 50°C, followed by extension of the primers for 3 minutes at 72°C. This cycle was repeated 25 times, resulting in specific IGF-I receptor fragments.

The isolated cDNA fragments were digested with the endonucleases BamHI and XbaI (New England Biolabs, MA, USA) and subcloned into the PBSII vector (Stratagene, CA, USA) by the method described by Sambrook et al., op. cit. Cells of E. coli strain MC1061 (T.V. Huynk et al., in DNA Cloning, Vol. 1 (D.M. Glover, ed.), IRL Press Ltd., Oxford, England, 1983, pp. 56-110) and SCS-1 (D. Hanahan, J. Mol. Biol. 166, 1983, pp. 557-580) were made competent according to the method described by D. Hanahan, in DNA Cloning, Vol. 1, supra, pp. 110-135, and used for transformation with the vectors indicated above. The soluble IGF-I receptor cDNA was assembled from four subcloned cDNA fragments as shown in Fig. 2 (BamHI-PstI, PstI-Asp718, Asp718-PstI, PstI-XbaI) according to the method described by Sambrook et al., op. cit. The cDNA fragment(s) were sequenced by the enzymatic chain termination method described by F. Sanger et al., op. cit., using T4 DNA polymerase (Sequenase Kit, USB, Cleveland, Ohio, USA). The cDNA sequence of the soluble IGF-I receptor is shown in Fig. 3A-3E. The sequence was

identical to the published sequence (A. Ullrich et al., The EMBO Journal 5(10), 1986, pp. 2503-2512).

Construction of cDNA coding for a hybrid insulin-IGF-I receptor

cDNA coding for a hybrid receptor composed of the human insulin
5 receptor described above wherein the cDNA sequence coding for
exons 2 and 3 was replaced by the cDNA sequence coding for
exons 2 and 3 from the IGF-I receptor, was prepared by PCR as
described above. Primer 2 (shown above), which includes the
insulin receptor "compatible" restriction site EcoRI, was used
10 to generate an IGF-I receptor cDNA fragment corresponding to
the DNA sequence encoding exon 2 and 3 and the coding part of
exon 1 of the insulin receptor which was inserted into the
EcoRI site of the insulin receptor cDNA by the method described
by Sambrook et al., op. cit.. The sequence of the cDNA fragment
15 was established as described above, and the sequence of the
IGF-I receptor cDNA fragment inserted into the insulin receptor
was found to be identical with the published sequence (A.
Ullrich et al., op. cit.). The cDNA sequence of the hybrid
receptor is shown in Fig. 4A-4F.

20 The cDNA fragment encoding the hybrid insulin-IGF-I receptor
constructed as described above was inserted in the mammalian
expression vector Zem219b (described in DK Patent Application
No. 3023/88) which carries a gene conferring methotrexate
resistance to the host cell, and cDNA encoding the hybrid
25 receptor was transfected into BHK cells, resulting in the cell
line FCW 38-16. Resistant cells were selected with 0.4-2 μ M
methotrexate.

The hybrid receptor (sIGF-I-R.1-68) comprising an IGF-I
receptor in which the 68 N-terminal amino acids of the insulin
30 receptor α -subunit have replaced the equivalent IGF-I receptor
segment was constructed by means of restriction enzyme sites
similarly positioned in the two receptor cDNAs. The BamHI-XhoI
cDNA fragments were exchanged between the insulin receptor and

the IGF-I receptor resulting in sIGF-I-R.1-68. This hybrid cDNA was inserted into the mammalian expression vector pZem 219b and transfected into BHK cells, resulting in the cell line FCW 110.

During cultivation in 5% FCS, the cell line FCW 110 formed 5 grape-like clusters of loosely attached cells, forming many floating cells that stayed alive, indicating that this cell line may be adapted to grow in suspension with or without serum.

EXAMPLE 2

10 Growth performance of cells transfected with the hybrid insulin/IGF-I receptor

Materials and methods

Cell lines: BHK (Baby Hamster Kidney cells, Syrian Hamster)
FCW 38-16, according to Example 1,
15 hIR 12-14, BHK transfected with DNA coding
for the wild-type human insulin receptor.

Cells were grown in 1 μ M MTX (only the transformed cell lines), DMEM, 10% FCS, 1% PS, 1% Gln.

In a first experiment, 10⁴ cells from each cell line were
20 divided among 25 different 5 cm culture dishes and grown in 5
% FCS on day 0. The cells were counted from 4 different plates
every day. Two independent aliquots were sampled from each
plate; where appropriate, floating or loosely attached cells
were also counted in aspirated medium. Cells grown in this
25 growth curve experiment were propagated in standard medium for
growing mammalian cells.

In a second experiment, 10⁵ cells from each cell line were
divided among 25 different 5 cm culture dishes on day 0. The
medium was changed on day 1 to 0% FCS, but was supplemented

with 5 mg/l of human insulin. The cells were counted over the next days as described above.

Results

a) Growth in medium with 5% FCS

5 The growth curves for the three different cell lines in FCS-containing medium are shown in Fig. 5. Based on the counts from the first three days, the cell lines FCW 38-16 and hIR 12-14 show a doubling time of 9 hrs as opposed to 16 hrs for BHK cells.

10 This experiment was repeated the following week, but this time with additional cell counts on day 1 and 2. The growth curves corresponding to the cell counts are shown in Fig. 6.

When figures from the first two days are used to calculate the generation time, FCW 38-16 and hIR 12-14 double each 14.4 hr
15 and BHK doubles each 20.4 hr. All three cell lines had the same cell counts on day four, and were not counted further.

In the second week of experiment, all three cell lines had longer generation times; the ratio between the generation time for the transformed cell lines and BHK was 1.4 as compared to
20 1.7 for the first week.

b) Growth in serum-free medium supplemented with human insulin

Three growth experiments with all three cell lines in serum-free medium supplemented with human insulin were performed over three weeks, and cell counts from these three experiments were
25 averaged to generate the growth curves shown in Fig. 6.

BHK cells grown without serum and without added human insulin were counted for one week.

FCW 38-16 consistently grew to higher densities under these conditions, but ultimately died on day 8 - 9.

hIR 12-14 formed grape-like clusters of loosely attached cells, forming many floating cells that stayed alive for 2 - 3 days.

5 Conclusion

In two independent growth-curve experiments in serum supplemented with 5% FCS, the transformed cell lines showed a shorter generation time than BHK cells. At the end of each experiment all three cell lines showed the same cell density on 10 the plates.

Although the generation times obtained in both weeks of analysis are not the same, it may be concluded that the growth curves support the observed difference in growth rate of FCW 38-16 (and hIR 12-14) compared to BHK.

15 Under serum-free conditions (supplemented with insulin), the FCW 38-16 cell line performed far better than both the BHK and hIR 12-14 cell lines, supporting the utility of cells of the present invention expressing a hybrid insulin/IGF-I receptor.

CLAIMS

1. A mammalian cell containing a hybrid DNA insert which comprises a first DNA sequence encoding part of the extracellular domain of a first cellular receptor and a second DNA sequence encoding part of the extracellular domain of a second cellular receptor which is specific for a different ligand than the first cellular receptor.
2. A cell according to claim 1, wherein the first DNA sequence encodes an exon or a fragment thereof.
- 10 3. A cell according to claim 1, wherein the second DNA sequence encodes an exon or a fragment thereof.
4. A cell according to any of claims 1-3, wherein either the first or the second DNA sequence encodes a ligand-binding site of the first or second receptor.
- 15 5. A cell according to claim 1, wherein the DNA sequences coding for the first and second receptors exhibit a high degree of identity/homology in the organization and/or structure of the exons encoded by said sequences.
6. A cell according to any of claims 1-5, wherein a DNA sequence encoding one or more exons, or a fragment thereof, of the extracellular domain of the first receptor is replaced by a DNA sequence encoding the corresponding exon or exons, or fragment thereof, of the extracellular domain of the second receptor.
- 20 7. A cell according to any of claims 1-5, wherein a DNA sequence encoding one or more exons, or a fragment thereof, of the extracellular domain of the second receptor is replaced by a DNA sequence encoding the corresponding exon or exons, or fragment thereof, of the extracellular domain of the first receptor.
- 30

8. A cell according to any of claims 1-7, wherein the first receptor is the insulin receptor.
9. A cell according to any of claims 1-8, wherein the second receptor is the insulin-like growth factor (IGF) receptor.
- 5 10. A cell according to any of claims 1-7, wherein the first receptor is the platelet-derived growth factor (PDGF) receptor.
11. A cell according to claim 10, wherein the second receptor is the epidermal growth factor (EGF) receptor.
12. A cell according to any of claims 1-7, wherein the first
10 receptor is the EGF receptor.
13. A cell according to claim 12, wherein the second receptor is the transforming growth factor (TGF) receptor.
14. A cell according to any of claims 1-7, wherein the first receptor is the TGF receptor.
- 15 15. A cell according to claim 14, wherein the second receptor is the EGF receptor.
16. A cell according to any of claims 1-7, wherein the first receptor is a growth hormone receptor.
17. A cell according to claim 16, wherein the second receptor
20 is a prolactin receptor.
18. A cell according to any of claims 1-7, wherein the first receptor is a prolactin receptor.
19. A cell according to claim 18, wherein the second receptor is a growth hormone receptor.

20. A cell according to claim 8, wherein a DNA sequence encoding exon 2, or a fragment thereof, of the insulin receptor is replaced by a DNA sequence encoding exon 2, or a fragment thereof, of the IGF receptor.

5 21. A cell according to claim 8, wherein a DNA sequence encoding exon 3, or a fragment thereof, of the insulin receptor is replaced by a DNA sequence encoding exon 3, or a fragment thereof, of the IGF receptor.

22. A cell according to claim 8, wherein a DNA sequence
10 encoding exons 2 and 3, or a fragment thereof, of the insulin receptor is replaced by a DNA sequence encoding exons 2 and 3, or a fragment thereof, of the IGF receptor.

23. A cell according to claim 9, wherein a DNA sequence encoding exon 2, or a fragment thereof, of the IGF receptor is
15 replaced by a DNA sequence encoding exon 2, or a fragment thereof, of the insulin receptor.

24. A cell according to claim 9, wherein a DNA sequence encoding exon 3, or a fragment thereof, of the IGF receptor is replaced by a DNA sequence encoding exon 3, or a fragment
20 thereof, of the insulin receptor.

25. A cell according to claim 9, wherein a DNA sequence encoding exons 2 and 3, or a fragment thereof, of the IGF receptor is replaced by a DNA sequence encoding exons 2 and 3, or a fragment thereof, of the insulin receptor.

25 26. A cell according to claim 22, wherein the DNA insert has the partial DNA sequence shown in Fig. 4A-4F (encoding the extracellular domain of the hybrid insulin/IGF-I receptor), or a suitable modification thereof.

27. A cell according to any of claims 1-26, which further contains an inserted DNA sequence encoding a desired polypeptide.

28. A process for producing a desired polypeptide, which
5 comprises culturing a cell according to claim 27 in a suitable nutrient medium under conditions conducive to the expression of the polypeptide and recovering the polypeptide from the culture.

29. A process according to claim 28, wherein the medium is a
10 serum-free medium.

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ACCGGAGCGCGCTCTGTATCGAGGAGACCCCGCTCCCGCAGCCATGGGACCGGG      60
TGCCCCCTCGCGCGAGACTAGGCTCTCTGGGCGCGAGGGCGTCGGTACCGCTGGCCCC
GCGCGGCGGGGGGCGCGCGCGCTGCTGGTGGCGGTGGCGCGCTGTCTACTGGGC      120
CGGCGCGCCCCCGCGCGCGCGGCGGACGACCCACCGCGCGGCGACGATGACCCG
GCGCGGCGCCACCTGTATACCCCGGAGAGGTGTCCCGGCATGGATATCCGGAACAACCTC      180
CGGCGCGCGGTGGACATGGGCGCTCTCCACAGGGCGGTACTATAGGCGTTGTGGAG
ACTAGGTTGCATGAGCTGGAGAAATGCTCTGTCAATCGMAGGACACTTGCAGATACTCTTG      240
TGATCCAACTACTCTGACCTCTTAACGAGACAGTAGCTTCTGTGAACTCTATGAGAAC
ATGTTCAAAACGAGGCCCGAAGANTTCCGAGACCTCAGTTTCCCAAACCTCATCATGATC      300
TACAGTTTTTGTCCGGGCTCTTAAAGGCTCTGGAAGTCAAGGGGTTTGAGTAGTACTAG
ACTGATTACTTGTCTCTTCCGGGCTATGGGCTCGAGAGCTGGAAGGACCTGTTCGCC      360
TGACTAATGAAACGACGAGAAGGCCAGATACCCGAGCTCTCGGACTTCTCTGCAAGGGG
AACTCAGGCTCATCGGGGATCAGGACTGTCTTTAACTACGGGCTGGTCACTTTCGAG      420
TTGGAGTGCAGTAGGCCCCCTAGTGTGACAAAGAAATTGATGCGCGACCCAGTAGAAGCTC
ATGTTTCACTCAAGGAACTCGGGCTCTACAACTGATGAACATCACCGGGGTTCTGTG      480
TACCAGTGGAGTTCCTTGAGCGGAGATGTGGACTACTTGTAGTGGGCCCCAAGACAG
CGCATCGAAGAAACAATGAGCTCTTTTACTTGGCCACTATCGACTGGTCCGTAATCTTG      540
GCGTAGCTCTTCTTGTACTCGAGACAATGAACCGGTGATAGTACACAGGGCATAGGAC
GATTCGTTGGAGGATAAATTACATCGTGTGTGAACAAGATGACAACGAGGAGTGTGGAGAC      600
CTAAGGCACCTCTATTAAATGAGCACAACTTGTTTTCTACTGTCTCTCACACCTCTG

```

Fig. 1a

2/30

601 ATCTGTCGGGTACCGGAAGGCAAGACCAACTGCCCCCGCCACCTCATCAACGGGCAG
 TAGACAGGCCCATGGCGCTTCCGGTTCTGGTTGACGGGGCGGTGGCAGTAGTTGCCCGTC
 661 TTGTCGAACGATGTTGGACTCATAGTCACTGTCAGAAAGTTTCCCGACCACTGTAAAG
 AAACAGCTTGCTACAACCTGAGTATCATGTGACGGTCTTTCAACGGGCTGGTAGACATTC
 720 TCACAGGCTGCACCGCGAAGGCCTCTGTGTGCACAGCGAGTGCCTGGGCAACTGTTCCT
 AGTGTGCGAGGTGGCGGCTTCCGGGACAAACGGTGTGCTCA CGGACCCGTTGACAAGA
 781 CAGCCGAGCAGACCCACCAAGTGCCTGGCGCTGCCGCAACTTCTACCTGGACGGCAGGTGT
 GTGCGGGTCTGGGGTGTGTCA CGACCGGACGGCGTTGAAGATGGACCTGCGCGTCCACA
 841 GTGGAGACCTGCGCCCGCTACTACACTTCCAGACTGGGGCTGTGTGAACCTTCAGC
 CACCTCTGGACGGGCGGGGCGATGATGTGAAGTCTCTGACCGGACACACTTGAAGTCG
 901 TTCTGCCAGGACCTGCAACCAAMTGCAGAACTGCGGAGGGCAGGGCTGCCACCATAC
 AAGACGGTCTGGACGTGGTGTGTTAGTGTCTTGAGGGCTTCCGTCGACGGTGGTCAATG
 961 GTCAATTCACAACAAGTGCATCCCTGAGTGTCCCTCCGGGTACACGATGAATTCAGC
 CAGTAAGTGTGTGTTACGTAGGACTCACAGGGAGGCCCATGTGTCTACTTAAGGTCC
 1021 AACTTGTCTGCAACCCATGCTGGGTGCTGTGTC CCAAGGTGTGCCACCTCTTAGAAGGC
 TTGAACACACA CGTGGGTGTACGGACCCAGGACAGGGTTCCACACGGTGGAGGATTTCCG
 1081 GAGAGACCATCGACTCGGTGACGTTGCCCCAGAGCTCCGAGGATGCACCGTCATCAAC
 CTCCTTGTGTAGCTGAGCCACTGACAGCGGGTCTCTGAGGCTCTTACGTGGCAGTAGTTG
 1141 GGGAGTCTGATCATCAACATTGAGGAGGCAACAACTGTGGCAGCTGAGCTAGAGCCCAAC
 CCTTCAGACTAGTAGTTGTAAGCTCTCTCGTGTGTAGACCGTGCATCGATCTTCGGTTG

Fig. 1b

REPLACEMENTSHEET

3/30

1201 CTCGGCTCATTGAGAAATTCAGGATCTATAAATCCGCCATCCTACGCTCTGGTG 1260
 GAGCCGGAGTAATCTTTTAAAGTCCCATAGATTTTATAGCGGCTAGGATCGAGACCAC
 1261 TCATCTTCTCTTCCTCGGAAGTTAGCTGTGATTCGAGGAGAGACTTGAATTTGGGAC
 AGTGAAGGAGAAAGGCTTCAATGAGACTAGCTCTCTGGAACCTTTAACCCCTTG 1320
 TACTCTTCTATGCTTGGACAACAGAACTAAGCAGCTCTGGAGCTGGAGCAAAACAC 1380
 ATGAGGAGATACGGAACTGTGTGCTTGGATTCCGTCGAGACCTGACCTCGTTTGTG 1380
 1321 AACCTACCATCACTCAGGGGAAACTCTTCTCCACTATPAACCCCAACTCTGCTTTGTCA 1440
 TTGGAGTGTAGTGATGCTCCCTTTGAGAGAGAGGTGATTTGGGGTTTGAGAGCAAGT 1440
 1441 GAATCCACAAGATGGAAGAAGTTTCAGGAACCAAGGGGGCGCAGGAGAGAAACGACTT 1500
 CTTTAGGTGTCTACTTCTTCAAGTCTTGGTTCCTCCCGGGTCTCTCTTTTGTCTGTA 1500
 1501 GCCCTGAAGACCAATGGGACGAGGCACTCTGTGAATATGAGTTACTTAAATTTCTTAC 1560
 CGGACTTCTGGTTACCCCTGGTCCGTAGACACTTTTACTCAATGAATTTAAAGAATG 1560
 1561 ATTCGGACATCTTTTGACAAGATCTTGTGTAGATGGGAGCGTACTTGGCCCCCGGACTTC 1620
 TAGCTGTAGAAACTGTCTTAGAAGACTCTACCTCTGGCATGACCGGGGGGCTGAAG 1620
 1621 CGAGACTCTTGGGGTTCATGCTTTCTACAAAGAGGCCCTTATCAGAAATGTGACGGAG 1680
 GCTCTGAGAACCCCAAGTACACAAGATGTTCTCCGGGAATAGTCTTACACTGSCCTC 1680
 1681 TTGACGGGCGAGGATGCATGTTGTTCCACAGTTGGAGCGTGGTAGACATGACCCACCC 1740
 AAGTGTCCCTCTACGTACCAAGGTTGTCAACCTCCACCATCTGTACTGSGGTGG 1740
 1741 CTGAGGTCCACGACCCCAATCAAGAACCAACCCAGGTGGCTGATCGGGGTCTCAAG 1800
 GACTCCAGGTGCTGGGTTTAGTGTCTTGGTGGTCCACCCGACTACGCCCGGAGTTC 1800

Fig. 1c

4/30

1801 CCCTGGACCCAGTATGCCATCTTTGTGAAGACCTGGTCACTTTTTCGGATGAACGCCG + 1860
 GGGACCTGGGTACAGGTAGAAACACTTCGGGACCACTGGGAAGCCTACTTTCGGCC
 1861 ACCTATGGGCAAGAGTGACATCATTTATGTCCAGACAGATGCCACCAACCCCTCTGTG + 1920
 TGGATACCCCGTTCTCACTGTAGTAATAACAGGCTCTCTACGGTGGTTGGGAGACAC
 1921 CCCCTGGATCCCAATCTCAGTGTCTAACTCATCCAGATTATCTGAAGTGGAACCA + 1980
 GGGGACCTTAGGTTAGAGTCACAGATTGAGTAGTAGGGCTTAATAAGACTTCACCTTTGGT
 1981 CCCTCCGACCCCAATGGCAACATCACCTACCTGGTTTTCGGAGAGGCAGCGGAA + 2040
 GGGAGGCTGGGTTACCGTTGTAGTGGGTGATGGACCAAGACCCCTCTCCGTCGCGCTT
 2041 GACAGTGAGCTGTTTCGAGCTGGATTATTGCTCAAGGGCTGAAGCTGCCCTCCGAGACC + 2100
 CTGTCACTCGACAAGCTCGACCTAATAACGGAGTTTCCCGACTTCGACGGGAGCTCCTGG
 2101 TGGTCTCCACATTTCGAGTCTGAAGATTCTCAGAAGCAACCCAGAGTGAGTATGAGGAT + 2160
 ACCAGAGGTGTAAGCTCAGACTTCTAAGAGCTTCTGTTGGTCTCACTCATACTCTTA
 2161 TCGGCCGGGGAATGCTCTCTGTCCAAAGACAGACTCTCAGATCCTGAAGAGAGCTGGAG + 2220
 AGCCGGCCGTTACGACGAGGACAGGTTTCTGTCTGAGAGCTTAGGACTTCTCTCGACCTC
 2221 GAGTCTCTGTTTAGGAAGACGTTTGAGGATTACCTGCACACGTTGGTTTTCGTCCCCAGG + 2280
 CTCAGAGCAAAATCTTCTGCAAACTCTTAATGACGTGTTGCCACAAAGACAGGGGTCC
 2281 CCACTCGGAAACGGAGTCCCTTGGCGATGTTGGGAATGTGACGGTGGCCGTCGCCACG + 2340
 GGTAGAGCCTTTTGGTCCAGGAACCGCTACAAACCTTACACTGCCACCCGGCAGGGTGC

Fig. 1d

5/30

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2341 GTGCAGCTTTCCCAACACTTCTCGAACGAGTGCCCAACGAGTCGGGAGGACACAGG+ 2400
      CACCGTCGAAAGGGTGTGAAGGAGCTGTGCGACGGGTGCTCAGGCTCTCTCGTGCC
      CCTTTTGAGAAAGTGTGAACAGGAGTGCCTGGTCACTCTCGGCTTGCGACACTTCAGG+ 2460
      GGAAGAACTCTTTCCACCACTTGTTCCTCAGCGACCAAGTAGAGGCCGACGCTGTGAAGTGC
      GGCTATCGCATCGAGCTGCAGGCTTGCAACAGGACACCCCTGAGGAACGGTGCAGTGTG+ 2520
      CCGATAGCTAGCTCGACGTCGCAACGTTGCTCTGTGGGACTCTTGCACGTCACAC
      GCAGCCTACGTCAGTGCAGGACCAATGCTGAGCCAGGCTGATGACATTTGTTGGCCTT+ 2580
      CGTCGGATCGAGTCACGCTCTGCTGACGGACTTCGGTTCGGACTACTGTACACACCGGA
      GTGACGATGAATCTTTGAGAACACGCTGCTCCACTTGATGTGCGAGAGCGCGAGGAG+ 2640
      CACTGCGTACTTTAGAAACTCTTGTGCGAGGTGAACACACCGTCTCGGCTTCTC
      CCCAATGGTCTGATCGTGTCTGATGAGTGAGTTATCGGCGATGTGTGATGAGGAGCTG+ 2700
      GGGTTACGAGACTAGCACACATCTTCACTCAATAGCCCTATACCACTCTCTCGAC
      CATCTTCGACACCCGACAGCACTTCGCTCTGGAAACGGGGCTGCAGGCTGCGTGGGCTG+ 2760
      GTAGAGACGCTGTGGGCGTTCGTGAGGAGACACTTTCGCCGACGTCGAGACCCGAC
      TCACCGGGGAACATACAGCGTSCGAATCCGGGCCACCTCTTCGCGGGCAACGGCTCTTGG+ 2820
      ATGGCCCCCTTGATGTGCAACGCTTAGGCCCGGTGGAGGGAACGCCCTTGGCGAGAAC

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Fig. 1e

6/30

2821 ACGGAACCCACCTATTCTACGTGACAGACTATTAGAGTCCCGTCAATATTGCAAAA
 TGCCTGGGTGGATAAAGATGCATCTGTGATAAATCTGCAGGCGATTTATACGTTTTT
 2880
 2881 ATTATCATCGGCCCCCTCATCTTTGTCTTTCTCTTCAGTGTGTGATTGGAAGTATTAT
 TAATAGTAGCCGGGGAGTAGAAACAGAAAGAGAAGTCAACAACACTAACCTTCATAAATA
 2940
 2941 CTATTCTGGAAGAAGAGCCAGATGGCGCTGGGACGCTTTACGCTTCTTCAAC
 GATAGGACTCTTTCTCCGTCGGTCTACCCGGCGACCTTGGCGAAATGGGAAGAAGTTTG
 3000
 3001 CCTGAGTATCTCAGTGCCAGTGATGTGTTTCCATGCTCTGTGTAGTGGCGGAGGTGG
 GGACTCATAGAGTACGGTCACTACACAAAGGTACGAGACACATGCACGGGCTGCTCACC
 3060
 3061 GAGGTGCTCTCGAGAGAAGATCACCTCTTCGAGAGCTGGGCGAGGCTCTCTTGGCATG
 CTCACAGAGCTCTCTTCTAGTGGGAGGAGCTCTCGACCCGCTCCGAGGAGCGCGTAC
 3120
 3121 GTGTATGAGGGCAATGCCAGGGACATCATCAAGGGTGAGGCGAGAGCCGCGTGGCGTG
 CACATACTCCCGTTACGGTCCCTGTAGTGTCTCCCACTCCGCTCTGGGGGACCGCCAC
 3180
 3181 AAGACGGTCAACGAGTCAGCGAGTCTCCGAGAGCGGATGAGTTCCTCAATGAGGCTCG
 TTCTGCCAGTTGTCTAGTGGTCAGAGGCTCTCGCTTAATCAAGGAGTTACTCGGAGC
 3240
 3241 GTCATGAGGGCTTCACTGCGCATCACTGGTGCGCTCTGGGAGTGTGTCCAGGGG
 CAGTACTTCCCGAAGTGGACGGTATGCAACCGGCGAGGACCTTCAACAGGTTCCCG
 3300
 3301 CAGCCACGCTGTGTGTGATGGAGCTGATGGCTCACGGAGACCTGAGAGCTACTCTCGT
 GTCGGGTGGACCAACCTACTCTGACTACCGAGTGGCTCTGGACTTCTCGATGAGGCA
 3360
 3361 TCTCTGGGCGAGAGGCTGAGATATCTTGGCGCGCTCTCCCTTACCTTTCAGAGATG
 AGAGAGCGCGGTCTCCGACTCTTATTAGGACCGGGGAGGGGATGGGAAGTTCTCTAC

Fig. 1f

7/30

3421 ATTCAGATGCGCGCAGAGATTGCTGACGGATGGCTTACCTGAACGCCAAGAAGTTTGTG
 3480 TAAAGTCTACCGCGCTCTTAACGACTGCCCTACCGGATGGACTTCCGGTCTTCTCAACAC
 3481 CATCGGACCTGGCAGCGAGAACTGCATGGTCGCCCATGATTTTACTGTCAAAATTGGA
 3540 GTAGCCCTGGACCGTCGCTCTTTGACGTACACAGCGGGTACTAAAATGACAGTTTAACTT
 3541 GACTTTGGAAATGACACAGAGACATCTATGAACGGATTACTACGGAAAGGGGCAAGGCT
 3600 CTGAAACCTTACTGGTCTCTGTAGATATTTTGCCTTAATGATGGCTTTTCCCCGTTCCCA
 3601 CTCTCCCTGTACGGTGGATGGACCGGAGTCCCTGAAGGATGGGGTCTTCCACCACTTCT
 3660 GACGAGGGACATGCCACCTACCGTGGCTCAGGGACTTCTTACCCGAGANGTGGTGAAGA
 3661 TCTGACATGTGGTCTTTTGGCGTGGTCTTTTGGGAAATCACCAGCTTGGCAGAACAGCCT
 3720 AGACTGTACACAGGAAACCGCAC CAGGAAACCTTTTGTGGTGGAAACCGTCTTGTGCGA
 3721 TACCAAGGCTGTCTAATGAACAGGTGTGAAATTTGTCAATGGATGGAGGTATCTGGAT
 3780 ATGGTTCCGGACAGATTACTTGTCCACAACTTTTAAACAGTACTACCTCCCAATAGACCTA
 3781 CAACCCGAACTGTCCAGAGAGAGTCACTGACCTCATGGCGATGTGCTGGCAATTCAC
 3840 GTTGGGCTGTTGACAGGTCTCTCTCAGTGACTGGAGTACCGGTACACGACCGTTAAGTTG
 3841 CCCAACAAGAGGCAACCTTCTGGAGATTGTCAACCTGCTCAAGGACGACCTGCACCC
 3900 GGGTTGTACTCCGGTTTGAAGGACCTCTAACAGTTGGACGAGTCTCTGTGCGACGTGGGG
 3901 AGCTTTCCAGAGGTGCTGTTCTTCCACAGCGAGGAGAACANGCTCCCGAGAGTGAAGAG
 3960 TCGAAGGCTCTCCACAGCAAGAGGTGTCGCTCTCTTGTTCGAGGGCTCTCACTCTTC
 3961 CTGGAGATGGAGTTTGGAGCATGGAGAAATGGCCCCCTGGACCGTTCTTCGCACTGTCAAG
 4020 GACCTTACTCTAACTCTCTTACTCTTACACGGGGACCTGGGAGAGCGCTGACAGTC

Fig. 1g

REPLACEMENT SHEET

40201	AGGAGGAGCGGGGGCGGGGATCGAGGGCTCTCGCTGGGTTTTCAGCGGAGCTACGAG	4080
	TCCTCTCTCGCCCCCGGCTTACCCTCCAGGAGCGACCCAAAGTTTCGCTCGTGCCTC	
40801	GAAACATCTCCTTACACACATGAACGGAGGCGAAGAAAAAGGGCGGATCTGTGACCTTG	4140
	CTTGTGTAGGAAATGTGTGTACTTGTCCCTCGTTCTTTTGTCCCGCTAAAGACTGGAAAC	
41401	CTCGGTCCAACTCTTCTACAGACTGCTACCGTGGCGGGGGCGGACGGGTGTCCCATTT	4200
	GGAGCCAGGTTAGGAAGGATGTTCACGGATGGCACCGCCCCGCTCCCGCAAGGGTAA	
42001	TTTCGTTTCTCTCGTTTGAAGGCTCTGGAANAATCTCAGAGATTCTCAGACTCTACACATG	4260
	AAGCGAAGGAGACCAAACTTTCCGAGACCTTTTGAAGTCTTAGAGAGTCTGTGAGTGGTAC	
42601	TCCAAATGGAGTTACAGAGATCGTTTCCATACATTTCTGTTCATCTTAAGGTGGACTCGTTT	4320
	AGGTTATCCTCAAGTCTCTAGCAAGGATATGTAAAGACAAGTAGAATTTCCACTGTAGCAAA	
43201	GGTTACCAATTTAACTAGTCTCTGCAGAGATTTAACTGTGAACCTGGAGGCGCAAGGGGTT	4380
	CCAAATGTTAAATATGATCAGACGCTCTCTAAATTTGACATCTTGACCTCCCGTTCGCCNA	
43801	TCCAGACTGTGCTGCTCTTTGGGCAACGACGGTTTCTAAACCCAGGATTTGTGTGTTTTTC	4440
	AGGTTGTCAAGCGGAAACCCCGTGTGTCTGCCNAATTTGGTCTTAAACCAAAAAG	
44401	GTTCCTCCCAACCGGCCCCGACGACATGGCAAGGAAGCAACCTGTGTTTACAAATCTTTTT	4500
	CAAGGGGGTGGCGGGGTGCTACTCTTCTCTTCTGTCGACAAAATGTTTAAGAAA	
45001	TTTTTTTTTTTTTTTCTGTGTGCTCGAGCTTCAGTATAAAGACAACCTCTCTGTTTG	4560
	AAAAAAMAAAAAAGCACACAGACTCGAAGTCAATATTTCTGTGTTTTGAAGGACAAC	
45601	TGGACAATAAGTTTCAAGAAAAAACAACAAAACAAAACACCCAGGCGCTGTTCAGAGAAAT	4620
	ACCTGTTTTCAAGCTTCTTTTTTGTGTTTTGTTTTGTTGGTGGGCAAGAGGCTCTTFA	

Fig. 1h

9/30

4621 TTCAGTTTTCAGGTGAGCTTCAAGTGGTTTTTTTGGTTTTTTTTTTTCTCTCATC
 +-----+-----+-----+-----+-----+-----+-----+-----+
 AAGTTCAAAATGCCAACTCGAAGTTCTACCAAAAACCAAAAAAAGAGAGTAG 4680
 CAGGCTGAAGGATTTTTTTTTTTTACAAAATGAGTTCTCAAAATGACCAATAGCTGC
 4681 GTCGCACTTCTAAAAAAGAAATGTTTTTCTCAAGGAGTTAACTGGTTATCGACG 4740
 TGTCTTCATATTTTGGATAAGGCTGTGGTCCCGGGGTGTGCTCACGTGTGTATGCAG 4800
 ACGAAAGTATAAAACCTATTCCAGACACCAAGGCCGCACAGAGTGCACACATACGTGC
 TGTGTGTCTCCATTAGACACGGCTGACGTGTGTGCAAAAGTATCCATCGGAGTTGATGCT 4860
 ACACACAGAGTAATCTGTGCCGACTGCACACACGTTTCATAGGTACGCTCAACTACGA
 TTGGGAATTGGCTCATGAAGGTTCTTCTCAAGGTTGCGAGCTCATCCCTCTCTCTCTTC 4920
 AACCTTAACCGAGTACTTCCAAGAGAGTTCCACGCTCGAGTAGGGGGAGAGAGGAG 4980
 CTTCTTATTGACTGGGAGACTGTGCTCTCGACAGATTTCTTGTGTGAGAAAGTCTAGCC
 GAAGAATAACTGACCTCTGACACGAGAGCTGTCTAAGAAGNACACAGTCTTCAGATCGG
 TCAGGTTTCTACCTCTCTTCCATTGGTGCCCAAGGGAGGAGCATTTCAATTGGAGTGA
 4981 AGTCCAAAGATGGGAGGAAGTGTAACCAACCGGTTCCTCTCGTAAAGTAAACCTCACT 5040
 TTATGAATCTTTTCAAGACCAACCAAGCTAGGACATTAATAAAAAAAGAAAGAAAGA 5100
 AATACTTAGAAAGTCTGGTTGGTTCGATCCTGTAAATTTTTTTTTTTTCTTTTCT 5160
 AAGAAAAAATAATGAAAAAGAAAAAAGAAAGAAAGAAAGAAAGAAAGAAAGAAAGAAAG
 5101 TTTCTTTTGGTTTACCTTTTCTCTTTTCTTTTCTTTTCTTTTCTTTTCTTTTCTTTTCT 5160
 ATATATTGTGACCATATTT
 5161 TATATAAACATGGTATAAA 5179

Fig. 1i

REPLACEMENT SHEET

10/30

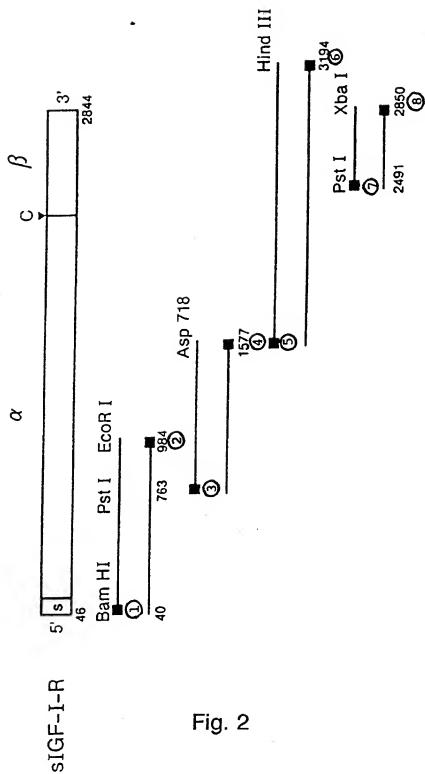


Fig. 2

12/30

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525 ATCTTCGATGACCAATCTCAAGGATNTGGGCTTTACACCTGAGGACAACTTACTCGG 584
      TAGAAGCTCTACTGGTTAGAGTCTCTATACCGGAAATGTGGGACTCTTGTAATGAGCC
      I F E M T N L K D I G L Y N L R N I T R -
585 GGGCCATCAGGATTTGAGAAAATGCTGACCTCTGTACTCTCCACTGTGGACTGTGTC 644
      CCCCCTGAGTCTTAACCTTTTACGCTGGAGACAAATGGAGAGGTGACACCTGACCAAG
      G A I R I E K N A D L C Y L S T V D W S -
645 CTGATCTCGATGCGGTGTCGAATACTACTATTTGTGGGGAATAGCCCCCAAGGATGT 704
      GACTAGGACCTAGCCACAGGTTATGTATGTAACACCCCTTATTCGGGGGTTTCTTACA
      L I L D A V S N N Y I V G N K P K E C -
705 GGGGACCTGTGTCAGGACCATGGAGGAGAGCCGATGTGTGAGAAGACCAACCATCAAC 764
      CCCCCTGGACAGAGTCCCTGTGACTCTCTTCGGCTACACACTCTCTGTGTGTAGTGG
      G D L C P G T M E E K P M C E K T T I N -
765 AATGAGTACAACTACGCTGCTGGACACAAACCGCTGCCAGAAATGTGCCCAAGCAG 824
      TTACTCATGTTGATGGCAGACCTGTGTGTGGGACGGCTTTTACACGGGTTCGTGC
      N E Y N Y R C W T T N R C Q K M C P S T -
825 TGTGGGAGCGGGGTGCACCGAGACAAATGAGTCTGCCACCCCGAGTGTCTGGGAGC 884
      ACACCTTCGCCCGCAGGTGCTCTTGTACTCAGACGGTGGGGCTCACGGACCGTCTG
      C G K R A C T E N N E C C H P E C L G S -

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Fig. 3b

REPLACEMENT SHEET

13/30

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885      TGCAGCGGCTGACAAAGCAGCGGCTGTGTAGCTTGCCGCCACTACTACTATGCCGGT      944
          ACGTGCGCGGACTGTTGCTGTGCGGGACACATCGAACGGCGGTGATGATACGGCCA
          C S A P D N D T A C V A C R H Y Y A G -
          GTGTGTGCTGCTGCGCCGCCCAACCTACAGGTTGAGGGCTGGCGCTGTGTGAC      1004
          CAGACACAGGACGGGCGGGTGTGGATGCCAACTCCGACCGCGACACACTG
          V C V P A C P N T Y R F E G W R C V D -
          CGTGACTTCTGCGCCAAACATCTCAGCGCGGAGCAGCGACTCCGAGGGGTTGTGATC      1064
          GCCTGAAGACGGGTTGTAGGAGTCGGGCTCTGCTGAGGCTCCCAACACTAG
          R D F C A N I L S A E S D S E G F V I -
          CACGAGCGGAGTCATGACGAGTGCCCTCGGGCTTCATCGCAAGCGCAGCCAGAGC      1124
          GTGTCGCCCTCAGTACGTCTCAGGGGAGCCGAGTAGGCGTTCGCCGTGGGTCTCG
          H D G E C M Q E C P S G F I R N G S Q S -
          ATGTACTGCATCCTGTGTAGGTCCTTGCCCGAGGCTGTGTAGGAAGAAAGAAACA      1184
          TACATGAGTAGGGAACACTTCCAGGAAGGGGCTCCAGACACTCTCTTTCTTTGT
          M Y C I P C E G P C P K V C E E K K T -
          AAGACCATGATTCGTACTTCTGTCTGATGCTCCAAGGTGCACCATCTTCAGGGC      1244
          TTCGTACTAGCAATGAAGCAGTCTACGAGGTTCCTACGTGGTAGAAGTTCCCG
          K T I D S V T S A Q M L Q G C T I F K G -
          AATTGCTCATATACATCCGACGGGGGAATACATTGCTTCAGAGCTGGAGACTTCATG      1304
          TTACAGGATTTGTAGGCTCCCTTTATTTGACGAGTTCGACCTCTTGAGTAC
          N L L I N I R R G N N I A S E L E N F M -

```

Fig. 3c

14/30

```

GGCTCATCGAGGTGACGGGTACGTGAAGATCGCCATTCTCATGCTTGGTCTCC 1305
CCCAGTAGTCTCACCACTGCCCGATGCACTTCTAGGCGGTAAGACTACGGACACGAGG 1364
G L I E V V T G Y V K I R H S H A L V S -
TTGTCTTCTTAAAAACCTTCGCCTCATCTAGGAGAGGAGCAGCTAGAGGGATTAC 1365
AACAGGAGGATTTTTTGAAGCGGAGTAGATCTCTCTCGTCGATCTTCCCTTAATG
L S F L K N L R L I L G E E Q L E G N Y -
TCTTCTACGTCTTCGACACACAGAACTTGCAGCACTGTGGACTGGGACCCGCAAC 1425
AGGAAGATGCAGAGCTGTGGTCTTGAAGCTCTTGACACCTGACCTGGTGGCGTTG
S F Y V L D N Q N L Q Q L W D W D H R N -
CTGACCATCAAGCAGGGAATGTACTTGTTCATTCCTCCAAATTATGTGTTCCGAA 1485
GACTGGTAGTTGCTCCCTTTACATGACGAAAGTTAGGTTTATACAAAGGCTT
L T I K A G K M Y F A F N P K L C V S E -
ATTACCCGATGGGAAGTGAAGGGGACTAAGGGCGCCAAAGCAAGGGGACATAAAC 1545
TAATGGCGTACTCTTCTACGTGCCCCGATTTCCCGCGTTTGGTTTCCCTGTATTG
I Y R M E E V T G T K G R Q S K G D I N -
ACAGAACACGGGGAGAGAGCTCTGTGAAGTGAAGTCTGTGCAATTACCTCCACC 1605
TGTGCTTGTGCCCCCTCTCTGAGGAGACACTTCTACTGAGGACGTAAAGTGAAGTGG
T R N N G E R A S C E S D V L H F T S T -
ACACCTCGAAGATCGCATCATATACCTGGACCGGTACCGGCCCTCTACTACAGG 1655
TGTGGACCTCTTACCGTAGTAGTATGGACCGTGGCCATGGCCGGGAGCATGATGCC
T T S K N R I I I T W H R Y R P P D Y R -

```

Fig. 3d

15/30

```

1725 GATCTCATCAGCTTACCGTTTACTACAGGAGCACCTTTAGAAATGTCACAGATAT 1784
      CTAGATAGTCGAAGTGGCAATGATGTTCTTCGTGGAAATCTTACAGTGTCTCATA
      D L I S F T V Y Y K E A P F K N V T E Y -
1785 GATGGCGAGGATGCTGCGGCTCCAAACAGCTGGAAACATGGTGGACGTGGACCTCCGCCC 1844
      TACCCGTCCTACGGACCGGAGGTGTCGACCTTGACCACTGCACCTGGAGGCGGG
      D G Q D A C G S N S W N M V D V D L P P -
1845 AACAGGACGTGGAGCCCGGCATCTTACTATGGGCTGAAGCCCTGGACTCAGTACGCC 1904
      TTGTTCTCGACCTCGGGCCGTAGAAATGATGATCCGACTTCGGGACCTGAGTCATCGG
      N K D V E P G I L L H G L K P W T Q Y A -
1905 GTTATCGTCAAGGCTGTGACCTCTACATGGTGGAGAACGACCATATCCGTGGGGCCAAG 1964
      CAATGCGATTCGACACTGGGAGTGGTACCACTCTTGCTGGTATAGGCACCCCGGTT
      V Y V K A V T L T M V E N D H I R G A K -
1965 AGTGAATCTGTACATTCGACCAATGCTTCAGTTCTCTTCATTCCTTTGGACGTTCTT 2024
      TCACCTAGAACATGTAAGCGTGTTACGAAGTCAAGGAGGTAAGGGAACCTGCAAGNA
      S E I L Y I R T N A S V P S I P L D V L -
2025 TCAGCATCGAATCTCTTCTCAGTTAATCGTAAGTGGAACTCCCTCTCTGCCCCAAC 2084
      AGTCGTAGCTTCAGGAGAAGAGTCAATTAGCACTTCACCTTTGGGAGGAGACGGGTTG
      S A S N S S S Q L I V K W N P P S L P N -

```

Fig. 3e

16/30

```

2085  GGCACCTGATGTTACTATGTGGCTGGCAGCGGACGCTCAGAGCGGTACTTTTAC      2144
      CCGTTGGACTCAATGATGTAACACGACCGCTCGCTCGGAGTCTGCCGATGAAATG
      G N L S Y Y I V R W Q R Q P Q D G Y L Y -
2145  CGGCACAATTACTGCTCCAAAGACAAAAATCCCATCAGGAAGTATGCCAGCGCACCATC      2204
      GCCGTGTTAATGACGAGGTTTCTGTTTAGGGGTAGTCTTCATACGGCTGCGGTGTAG
      R H N Y C S K D K I P I R K Y A D G T I -
2205  GACATTGAGGAGGTACAGAGAACCCCAAGACTGAGGTGTGTGGTGGGAGAAAGGGCCT      2264
      CTGTAACCTCTCCAGTGTCTCTTGGGGTCTGACTCCACACACACCCCTCTTTCCGGA
      D I E E V T E N P K T E V C G G E K G P -
2265  TGTGGCGCTGCCCCAAAACTGAAGCCGAGAGCAGGCCGAGAGGAGGAGGCTGAATAC      2324
      ACGACGCGAGCGGGGTTTGACTTCGGCTCTTCGTCGGGCTTCTCCTCCGACTTATG
      C C A C P K T E A E K Q A E K E E A E Y -
2325  CGCAAAGTCTTGAGAAATTCCTGCACAACTCCATCTTCGTGCCAGACCTGAAGGAAG      2384
      GCGTTTCAGAACTCTTAAAGGACGTGTGAGGTAGAAGCAGGCTGTGACTTTTCCTTC
      R K V F E N F L H N S I F V P R P E R K -
2385  CGGAGAGTGTATGCAAGTGGCCAAACACACCATGTCTCCAGCGGAAGCAGGAACACCG      2444
      GCCTCTCTACAGTACGTTCCACGGTTGTGGTACAGGTCCGGCTTCGTCTTGTTGGTGC
      R R D V M Q V A N T T M S S R S R N T T -

```

Fig. 3f

REPLACEMENT SHEET

17/30

```

2445  GCGCAGACCTACAACATCACGACCGGAAGAGCTGGAGACGAGTACCCTTCTTTT 2504
      CGGCTCTGTGGATGTTGTAGTGGCTGGGCTTCTCGACCTCTGTCTCATGGGAAGAA
      A A D T Y N I T D P E E L E T E Y P F -
2505  GAGCGACACTGGNTAACAGGAGAGAACTGTCTATTCTTAACCTTGGGCTTTCATTG 2564
      CTCTGCTCACCTATTGCTCTCTTGACAGTAGAGATTGGAGCCGGAAAGTGTAA
      E S R V D N K E R T V I S N L R P F T L -
2565  TACCGCATCATATCCACAGCTGCAACACGAGGCTGAGAAGCTGGCTGCAGGGGCTCC 2624
      ATGGCGTAGCTATAGTGTGACGCTTGGTCTCGACTCTTCGACCCGACGTCGCGGAG
      Y R I D I H S C N H E A E K L G C S A S -
2625  AACTTCGCTTTGCAAGGACTATGCCCGCAGAGGAGCAGATGACATTTCTTGGGCCAGTG 2684
      TTGAAGCAGAAAGCTTCCTGATACGGGGCTTCTCTGTCTACTGTAAAGACCCGGTCA
      N F V F A R T M P A E G A D D I P G P V -
2685  ACCTGGAGCCAGGCTGAAAACCTCATCTTTTAAAGTGGCGGAACCTGAGAACTCC 2744
      TGGACCTCGGTCGCGACTTTTGAGGTAGAAAATTTACCGGCTTGGACTCTTAGGG
      T W E P R P E N S I P L K W P E P E N P -
2745  AATGGATTGATTTCTAATGTATGAATAAATACGATCACAAGTTGAGGATCAGGAGAA 2804
      TTACCTAACTAGATTACATACITTTATTTATGCTAGTGTTCACCTCCTAGTGCCTT
      N G L I L M Y E I K Y G S Q V E D Q R E -

```

Fig. 3g

18/30

```

2805  TGTGTCTCAGACAGGAATACAGAAAGTATGGAGGGCCAGCTAAACGGCTAAACCCG
      +-----+-----+-----+-----+-----+-----+
2864  ACACACAGGTCTGTCTTATGTCTTCACTCACTCCCGGTTGCGATTGGCCGATTGGGC
      +-----+-----+-----+-----+-----+-----+
      C V S R Q E Y R K Y G G A K L N R L N P -
2865  GGGAACTACACAGCCCGGATTCAGGCCACATCTCTCTGGGAATGGGTCTGGACAGAT
      +-----+-----+-----+-----+-----+-----+
2924  CCCTTGATGTGTGGGCCTAAGTCCGGTGTAGAGAGAGACCTTACCAGCACCTGTCTA
      +-----+-----+-----+-----+-----+-----+
      G N Y T A R I Q A T S L S G N G S W T D -
      X
      b
      a
      I
2925  CCTGTGTTCTTCTATGTCTCAGGCCAAACAGGATATGAAAACCTAATCTAGAGGATC
      +-----+-----+-----+-----+-----+-----+
2980  GGACACAGAAGATACAGGTCCGGTTTGTCTTACTTTTGATTAGATCTCTTAG
      +-----+-----+-----+-----+-----+-----+
      P V F F Y V Q A K T G Y E N *

```

Fig. 3h

19/30

```

      B
      a
      m
      H
      I
160  ACCCGGGATCATGAAGTCTGGCTCCGGAGGAGGGTCCCGGACCTCGTGTGGGGCTCC 219
      TGGGCCCTAGGTACTTCAGACCGAGGCTCTCCAGGGGCTGGAGCGACACCCCGAGG
      M K S G S G G S P T S L W G L L -
220  TGTTCCTCTCGCGCGGCTCTGCTCTGGCCGACGAGTGGAGAAATCTCGGGGCCAGGCA 279
      ACAAAGAGCGCGCGGAGCGAGACCGGCTGCTCACCCTTTAGACGCCCGGTCGT
      F L S A A L S L W P T S G E I C G P G I -
280  TCGNATCGGCACGACTATCAGCAGCTGAGGGCCCTGGAGAACTGCACGGTGATCGAGG 339
      AGCTGTAGCGCTTGCTGATAGTGTGACCTTCGGGACCTTTGACGTGCCACTAGTCTC
      D I R N D Y Q Q L K R L E N C T V I E G -
340  GCTACTCCNCAATCTGCTCATCTCCAGGCGGAGGACTACGGAGCTACCGCTTCCCA 399
      CGATGGAGGTAGGACGAGTAGGTTCGGCTCCTGATGGGCTCGATGGCGAGGGGT
      Y L H I L L I S K A E D Y R S Y R F P K -
400  AGCTCAGGTCATTACCGAGTACTTCTGCTGTTCGAGTGGCTGGCTCGAGAGCCTCG 459
      TCGATGCCAGTATGGCTCATGACGACACAGGCTCACCGACGGAGCTCTCGAGG
      L T V I T E Y L L L F R V A G L E S L G -
460  GAGACCTCTTCCCMACCTACGGTCATCCGGCGGTGGAACTCTTCTACAACTACGCC 519
      CTCGTGAGAGAGGGTTGGAGTGCACGTAGGGCGGACCTTTGAGAAGATTTGATGGGG
      D L F P N L T V I R G W K L F Y N Y A L -

```

Fig. 4a

20/30

```

520 + TGGTCATCTCGAGATGACCAATCTCAAGGATATTGGGCTTTACAACCTGAGGAACATTA 579
      + ACCAGTAGAGCTCTACTGGTTAGAGTTCTTATAACCCGAAATGTTGGACTCTCTGTAAAT
      + V I F E M T N L K D I G L Y N L R N I T -
580 + CTCGGGGGCCATCAGATTGAGAAAAATCTGACCTCTGTTTACCTCTCTCACTGTGGACT 639
      + GAGCCCCCGGTAGTCTCTAACTCTTTTACGACTGGAGACAATGGAGAGGTGACACCTGA
      + R G A I R I E K N A D L C Y L S T V D W -
640 + GGTCCTGATCTGGATCGGTGTCCAATACTACATTGTGGGGAAATAGCCCCCAAGG 699
      + CCAGGGACTAGGACCTAGCCACAGGTTATTGATGTAACACCCCTTATTCGGGGGTTTC
      + S L I L D A V S N N Y I V G N K P P K E -
700 + AATGTGGGACTGTGTCCAGGGACCATGGAGGAGAGCCGATGTGTGAGAGACCACCA 759
      + TTACACCCCTGGACACAGGTCCCTGGTACCTCTCTTCGGCTACACACTCTCTCTGTGTGT
      + C G D L C P G T M E E K P M C E K T T I -
760 + TCACAAATGAGTACAACTACCGCTGTGGACCAACAAACCGCTGCAGAAATGTGCCAA 819
      + AGTTGTTACTCATGTTGATGCGGACGACCTGGTGTGTTGGCGACGGTCTTTTACACGGGTT
      + N N E Y N Y R C W T T N R C Q K M C P S -
820 + GCACGTGGGAAGCGGGCGTGCCACGAGAACAAATGATGTGCTGCCACCCCGAGTGCCTGG 879
      + CGTGCACACCCCTCGCCCGACGTGGCTCTTGTACTACGACGGTGGGGCTCACGGACC
      + T C G K R A C T E N N E C C H P E C L G -

```

Fig. 4b

REPLACEMENT SHEET

21/30

```

880  GCAGCTCAGCGCGCTGACACGACACGCGCTGTGTAGTCTGCCGCACTACTACTG
      CGTCGACGTCGCGGACTGTGTGTCGGACACATCGACGCGGTGATGATAC
      S C S A P D N D T A C V A C R H Y Y A -
940  CCGGTGTGTGTGCTGCGCTGCCGCCACACCTACAGGTTGAGGGTGGCGCTGTG
      GGCACAGACACACGACGACGCGCGGTGTGGATGCCAACTCCCGACCGCGAC
      G V C V P A C P P N T Y R F E G W R C V -
1000 TGGACCTGACTTCTGCGCCACATCTCAGCGCCGAGCAGCGACTCCGAGGGTTG
      ACTGGCACTGAAGACCGGTGTGAGGATCGCGGCTCTGCTGAGGCTCCCONAAC
      D R D F C A N I L S A E S S D S E G F V -
1060 TGATCCAGACGGCGATGATGACAGAGTGCCCTCGGGCTTCATCCGGATTCACGA
      ACTAGGTGCTGCGCTCAGTAGTCTCAGCGGAGCCCGAGTAGGCTTAAGTGCT
      I H D G E C M Q E C P S G F I R N S S N -
1120 ACTTGCTGTGACCCCATGCTGGGTCCCTGTCCAGGTGTGCCACCTCTAGAGGCG
      TGACGACACGTGGGTACGGACCCAGGACAGGTTCCACACGGTGGAGGATCTCCGC
      L L C T P C L G P C P K V C H L E G E -
1180 AAGACCATCTGACTGGTGATGCTGCCAGGAGCTCCGAGGATGCACCTCATACG
      TCTTGTGTAGCTGAGCCACTGCACGGGTCTCGAGGCTCTACGTGGCAGTAGTTC
      K T I D S V T S A Q E L R G C T V I N G -

```

Fig. 4c

REPLACEMENT SHEET

22/30

```

1240  GGAGTCTGATCATCAATTCGAGGAGGCAACAATCTGCAGCTGAGCTAGACGCAACC 1299
      +-----+-----+-----+-----+-----+-----+
      CTTACAGTAGTAGTTGTAAGCTCTCCGTTGTTAGACCGTGACTCGATCTTCGGTTGG
      S L I N I R G N N L A A E L E A N L -
1300  TCGGCTTCATTGAGAAATTCAGGGTATCTAAAAATCCGCCGATCCTACGCTCTGGTGT 1359
      +-----+-----+-----+-----+-----+-----+
      AGCGGAGTAATCTTTAAAGTCCATAGATTTTTAGCGCGCTAGGATGCGAGACCACA
      G L I E I S G Y L K I R S Y A L V S -
1360  CACTTTCCTTCCGAGACTTACTCTGATTCGAGGAGACCTTGGANATTGGAACT 1419
      +-----+-----+-----+-----+-----+-----+
      GTGAAGAGAGAGCGCTTCAATGCAGACTAAGCTCTCTTGGAACTTTAACCTTGA
      L S F R K L R L I R G E T L E I G N Y -
1420  ACTCTTCTATGCCCTTGGACACAGAACCTAAGCGAGCTCTGGGACTGGAGCAACACA 1479
      +-----+-----+-----+-----+-----+-----+
      TGAGGAAGATACGGAACTGTGGTCTTGGATTCCGTCGAGACCTGACCTCGTTGTGT
      S F Y A L D N Q N L R Q L W D W S K H N -
1480  ACCTCACCACTCAGGGGAACTCTTCTTCCACTATACCCCAAACTCTGCTGTGTCAG 1539
      +-----+-----+-----+-----+-----+-----+
      TGGAGTGGTGGTGAGTCCCTTTGAGAGAAGGTGATATTGGGGTTTGAGAGCAACAGTC
      L T T T Q G K L F F H Y N P K L C L S E -
1540  AAATCCACAAGATGGAAGATTTCAGGAACCAAGGGGCCCAAGGAGAAACGACATTG 1599
      +-----+-----+-----+-----+-----+-----+
      TTAGTGTTCTTACTTCTTCAAAGTCTTGGTTCCTCCGGGGTCTCTCTTTGCTGTAACT
      I H K M E E V S G T K G R Q E R N D I A -

```

Fig. 4d

23/30

```

1600 CCTGAAGACCAATGGGACAAGGCATCTGTGAAATGAGTTACTTAATTTCTTACA
      GGAAGTTCTGGTTACCCCTGTTCCGTAGGACACTTTTACTCAATGAATTTAAAGAATGT
      L K T N G D K A S C E N E L L K F S Y I -
1660 TTCGACATCTTTTGACAAGATCTTGTGAGATGGAGCGGTACTGGCCCCCGCATTC
      AAGCTGTAGAAAACCTGTTCTAGAAGCACTTACCCTGGCATGACCGGGGGCTGAAGG
      R T S F D K I L L R W E P Y W P P D F R -
1720 GAGACTCTTGGGGTTTCATGCTGTTCTACAAGAGGCCCTTATCAGAATGTGACGAGT
      CTCTGGAGAACCCCAAGTACGACAAGATGTTCTCCGGGAATAGTCTTACACTGCCTCA
      D L L G F M L F Y K E A P Y Q N V T E F -
1780 TCGATGGCAGAGTGGGTGTTCCAAACAGTTGGACGTTGGTAGACATTTGACCCACCCC
      AGCTACCCGCTCTACGCACACCAAGGTTGCAACCTGCCACCATCTGTAACTGGGTGGGG
      D G Q D A C G S N S W T V V D I D P P L -
1840 TGAGTCCAAACGACCCCAATCAAGAACACCCAGGGTGGCTGATGGGGGTCTCAAGC
      ACTCCAGGTTGCTGGGGTTTAGTGCTTGTGGTGGTCCCAACGACTACGCCCCAGAGTTCC
      R S N D P K S Q N H P G W L M R G L K P -
1900 CTGGACCCAGATGCGATCTTTGTGAGACCCCTGGTCACTCTTTTCGGATGAACCGCGA
      GGACCTGGGTCAATACGGTAGAAACACTTCTGGGACCACTGGAAGAACCTACTTGGCGGCT
      W T Q Y A I F V K T L V T F S D E R T -

```

Fig. 4e

REPLACEMENT SHEET

24/30

```

1960  CCTATGGGCCAAGAGTGACATCATTTATGTCACAGATGCCACCAACCCCTCTGTGC
1980  +-----+-----+-----+-----+-----+-----+
1990  GGATACCCCGGTTCTCACTGTAGTAATACAGCTCTGCTACGGTGTGGGGAGACAG
2010  +-----+-----+-----+-----+-----+-----+
2020  Y G A K S D I I Y V Q T D A T N P S V P -
      B
      a
      m
      H
      I
2040  CCTGTGATCCANTCTCAGTCTCTACTCATCATCCAGATTTATCTGAAGTGGAAACCAC
2060  +-----+-----+-----+-----+-----+-----+
2070  GGAACCTAGGTAGTACAGATGAGTAGTAGGGTCTAATAGACTTCACCTTGGTG
      L D P I S V S N S S Q I I L K W K P P -
2090  CCTCCGACCCCAATGCCAAGCATCACCACCTACCTGGTTTCTGGAGAGCGAGGGAG
2110  +-----+-----+-----+-----+-----+-----+
2120  GGAGCTGGGGTTACCGTTGTAGGGGTGATGGACCAAGAACCCCTCTCGTCCGCTTC
      S D P N G N I T H Y L V F W E R Q A E D -
2140  ACATGAGCTGTTCGAGCTGGATTATTCCTCAAGGGCTGAAGCTGCCCTCGAGACCT
2160  +-----+-----+-----+-----+-----+-----+
2170  TGTCACTGCACAGCTCGACCTAATACGGAGTTTCCGACTTCGACGGGAGCTCTGGA
      S E L F E L D Y C L K G L K L P S R T W -
2190  GGTCTCCACCTTCGAGTCTGAAGTCTTCAGAGCACACACAGAGTGATGAGGATT
2210  +-----+-----+-----+-----+-----+-----+
2220  CCAGAGTGGTAACTCAGACTCTAAGAGTCTTCGTGTGGTCTCACTCATCTCTAA
      S P P F E S E D S Q K H N Q S E Y E D S -
2240  CGGCGGCGAATGCTGCTCTCTCAAGACAGACTCTCAGATCTCGAAGAGCTGGAGG
2260  +-----+-----+-----+-----+-----+-----+
2270  GCGGCGGCTTACGACGAGGACAGGTTCTGTCTGAGAGCTTAGGACTTCTCGACCTCC
      A G E C C S C P K T D S Q I L K E L E E -

```

Fig. 4f

REPLACEMENT SHEET

25/30

```

2320 AGTCTCGTTTAGGAGACGTTGAGGATTACCTGCACAACGTGTTTTTGTCTCCACGAA
      +-----+-----+-----+-----+-----+-----+
2379 TAGAGACAAATCCTTCTCAAACTCTAATGACGTGTTGCACCAAAAGCAGGGGTCTT
      +-----+-----+-----+-----+-----+-----+
      S S F R K T F E D Y L H N V V F V P R K -
2380 AAACCTCTTCAGGCACGTGTCGCCGAGGACCTTAGGCCATCTCGGAACGCAGGTCCCTTG
      +-----+-----+-----+-----+-----+-----+
2439 TTTGAGAACTCCGTGACCACCGGCTCTCTGGATCCGTAGAGCCTTTCGTCAGGGAAC
      +-----+-----+-----+-----+-----+-----+
      T S S G T G A E D P R P S R K R R S L G -
2440 CGCATGTTGGGAATGACGGTGGCCGTGCCACGGTGGCAGCTTTCGCCAACACTTCCT
      +-----+-----+-----+-----+-----+-----+
      CGCTACAACCTTTACACTGCCACCGGACGGGTGCCACCGTCGAAAGGGTTGTGAAGG
      +-----+-----+-----+-----+-----+-----+
      D V G N V T V A V P T V A A P N T S S -
2500 CGACACGGTGCCCGACGAGTCCGGAGGACGACGGCTTTTGAGAAGGTGTGAACAAGG
      +-----+-----+-----+-----+-----+-----+
2559 GCTGGTGCACGGGTGCTCAGGCCTCTCGTGTCCGGAAACTCTCCACCACTTGTTC
      +-----+-----+-----+-----+-----+-----+
      T S V P T S P E E H R P F E K V V N K E -
2560 AGTCGCTGTGTCATCTCCGGCTTGCGACACTTCACGGGCTATCGCATCGAGTCGAGCCTT
      +-----+-----+-----+-----+-----+-----+
      TCAGCGACCACTAGAGGCCGAAACGCTGTGAAGTCCCGATAGCTAGCTCGACGTCCGAA
      +-----+-----+-----+-----+-----+-----+
      S L V I S G L R H F T G Y R I E L Q A C -
2620 GCAACGAGACACCCCTGAGGAACGTCAGTGTGGCAGCTACGTCAGTGCAGGACCA
      +-----+-----+-----+-----+-----+-----+
2679 CGTTGGTCTGTGGGACTCTTTGCCAGCTCACACCCTCGGATGCGAGTCACGCTCTGGT
      +-----+-----+-----+-----+-----+-----+
      N Q D T P E E R C S V A A Y V S A R T M -

```

Fig. 4g

REPLACEMENT SHEET

26/30

```

2680  TGCCTGAAGCCAAAGGCTGATGACATGTGTGGCCCTGTGACGCA TGAATCTTTGAGACA 2739
      ACGGACTTCGGTTCCGACTACTGTAACAAACGGGACACTGCGTACTTTAGAAATCTTTGT
      P E A K A D D I V G P V T H E I F E N N -
2740  ACGTCGTCCACTTGATGTGCAGAGCCGAAGAGGCCCAATGGTCTGATCGTGTGTATG 2799
      TGCAGCAGTGAACACTACACCGTCTCTCGGCTTCTCGGGTTACCCAGACTAGCAGCATAC
      V V H L M W Q E P K E P N G L I V L Y E -
2800  AAGTGAGTTATCGCCGATATGGTATGAGAGCTGCATCTCTGCGTCTCCCGCAAGCACT 2859
      TTCACTCAATAGCCGCTATACCACTACTCTCTGACGTAGAGCGCAGAGGCGGTTCCGTGA
      V S Y R R Y G D E E L H L C V S R K H F -
2860  TCGCTCTGGAACGGGGCTGCAGGCTCGTGGGCTGTACACGGGGAACCTACAGCGTGCAG 2919
      AGCGAGACCTTGCCCCGACGTCCGAGGCACCCGACAGTGGCCCTTGATGTGCCACGCTT
      A L E R G C R L R G L S P G N Y S V R I -
2920  TCCGGGCCACTCTCCCTTTCGGGGCAAGCGCTTTGGACGGAAACCCACTATTCTACGTGA 2979
      AGGCCCGGTGAGGGGAACGCCGTTSCCGAGAACCTTGCCTTGGGTGGATAAGATGCACT
      R A T S L A G N G S W T E P T Y P Y V T -
      x
      b
      a
      I
2980  CAGACTATTAGAGCTCCGTCGAATATTGCAAAATACTTAGAGGACTGGGTGGCAT 3039
      GTCTGATAAATCTGCAGGCGAGTTTATACGTTTATTAGATCTCTAGACCCACCCTGA
      D Y L D V P S N I A K * S R G S G V A S -

```

Fig. 4h

REPLACEMENT SHEET

27/30

```

CCCTGTGACCCCTCCCCAGTGCCTCTCTGGCCCTGGAGTGGCCACTCCAGTGGCCACC 3040
+-----+-----+-----+-----+-----+-----+-----+-----+
GGGACACTGGGAGGGGTCAAGAGAGGACCGGACCTTCAACGGTGAGGTCAAGGGTGG 3099
+-----+-----+-----+-----+-----+-----+-----+-----+
L * P L P S A S P G P G S C H S S A H Q -
AGCCTTGCTCTAATAAATTAAATTAAAGTTGCATCATTTTGTCTGACTAGTGTCTCTCTAAT 3100
+-----+-----+-----+-----+-----+-----+-----+-----+
TCGNAACAGGATTATTTTAAATTCAACCTAGTAAACAGAGACTGATCCACAGGAAGATATTA 3159
+-----+-----+-----+-----+-----+-----+-----+-----+
P C P N K I K L H H F V * L G V L L * Y -
ATTATGGGCTGGAGGGGGTGTATGGAGCAAGGGCAAGTTGGGAAGACAACCTGTAGG 3219
+-----+-----+-----+-----+-----+-----+-----+-----+
TAATACCCCACTCCCCCAACCATACCTTCGTTCCCGTTCAACCTTCTGTGGACATCC
Y G V E G G G M E Q G A S W E D N L * G -
GCCTGGGGGTCTATTGGGAACCAAGCTGGAGTGCAGTGGGCACAACTTGGCTCACTGCA 3220
+-----+-----+-----+-----+-----+-----+-----+-----+
CGGACGCCCAAGATAACCTTTGGTTGCACCTCACGTCAACCGTGTAGAACCGAGTGACGT
L R G L L G T K L E C S G T I L A H C N -
ATCTCCGCTCTCTGGTTCAA 3280
+-----+-----+-----+-----+-----+-----+-----+-----+
TAGAGCGGAGGACCAAGTT 3300
+-----+-----+-----+-----+-----+-----+-----+-----+
L R L G S S -

```

Fig. 4i

28/30

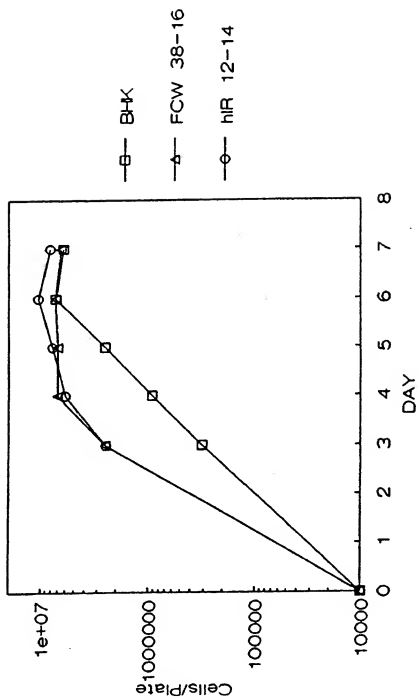


Fig. 5

29/30

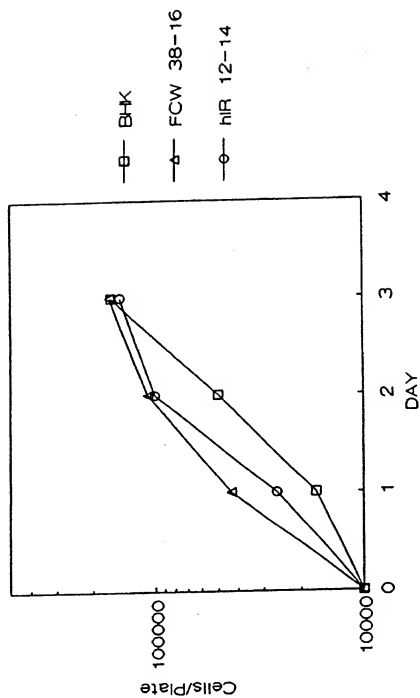


Fig. 6

30/30

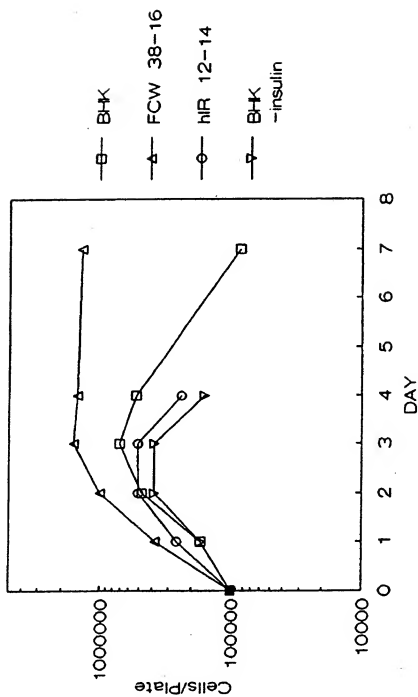



Fig. 7

INTERNATIONAL SEARCH REPORT

International Application No. PCT/DK 91/00116

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) ⁶		
According to International Patent Classification (IPC) or to both National Classification and IPC IPC5: C 12 N 15/62, 5/00		
II. FIELDS SEARCHED		
Minimum Documentation Searched ⁷		
Classification System	Classification Symbols	
IPC5	C 12 N; C 07 K; A 61 K	
Documentation Searched other than Minimum Documentation to the extent that such Documents are included in Fields Searched ⁸		
SE,DK,FI,NO classes as above		
III. DOCUMENTS CONSIDERED TO BE RELEVANT ⁹		
Category ¹	Citation of Document, ¹¹ with indication, where appropriate, of the relevant passages ¹²	Relevant to Claim No. ¹³
Y	Proc. Natl. Acad. Sci. USA, Vol. 83, November 1986 L. Ellis et al.: "Linking functional domains of the human insulin receptor with the bacterial aspartate receptor", see page 8137 - page 8141 ---	1-29
Y	WO, A1, 8905355 (THE SALK INSTITUTE FOR BIOLOGICAL STUDIES) 15 June 1989, see the whole document ---	1-29
Y	EP, A1, 0244221 (GENENTECH, INC.) 4 November 1987, see the whole document ---	1-29
Y	The Journal of Cell Biology, Vol. 109, November 1989 L. Sistonen et al.: "Activation of the neu Tyrosine Kinase Induces the fos/jun Transcription Factor Complex, the Glucose Transporter, and Ornithine Decarboxylase", see page 1911 - page 1919 ---	28-29
<p>* Special categories of cited documents:¹⁰</p> <p>"A" document defining the general state of the art which is not considered to be of particular relevance</p> <p>"E" earlier document but published on or after the international filing date</p> <p>"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another claim or other special reason (as specified)</p> <p>"O" document referring to an oral disclosure, use, exhibition or other means</p> <p>"P" document published prior to the international filing date but later than the priority date claimed</p> <p>"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</p> <p>"X" document of particular relevance, the claimed invention cannot be considered novel or cannot be considered to involve an inventive step</p> <p>"Y" document of particular relevance, the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such docu- ments, such combination being obvious to a person skilled in the art</p> <p>"&" document member of the same patent family</p>		
IV. CERTIFICATION		
Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report	
17th July 1991	1991 -07- 25	
International Searching Authority	Signature of Authorized Officer	
SWEDISH PATENT OFFICE	 Yvonne Stösteen	

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)		
Category *	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No
A	Science, Vol. 240, June 1988 B.K. Kobilka et al.: "Chimeric $\alpha 2$ -, $\beta 2$ -Adrenergic Receptors: Delineation of Domains Involved in Effector Coupling and Ligand Binding Specificity", see page 1310 - page 1316 --	1-29
A	EP, A2, 0325262 (THE GENERAL HOSPITAL CORPORATION) 26 July 1989, see the whole document --	1-29
A	Proc. Natl. Acad. Sci. USA, Vol. 84, August 1987 L. Ellis et al.: "Heterologous transmembrane signaling by a human insulin receptor-v-ros hybrid in Chinese hamster ovary cells", see page 5101 - page 5105 ----- -----	1-29

**ANNEX TO THE INTERNATIONAL SEARCH REPORT
ON INTERNATIONAL PATENT APPLICATION NO.PCT/DK 91/00116**

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report. The members are as contained in the Swedish Patent Office EDP file on 91-06-27. The Swedish Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
WO-A1- 8905355	89-06-15	AU-D-	2818889	89-07-05
		EP-A-	0325849	89-08-02
		US-A-	4981784	91-01-01
EP-A1- 0244221	87-11-04	JP-A-	62272990	87-11-27
		US-A-	4859609	89-08-22
EP-A2- 0325262	89-07-26	AU-D-	3281889	89-08-11
		WO-A-	89/06690	89-07-27